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DEFENCE R&D DÉFENSE

Inhalation Toxicity of Aerosolized Nerve Agents

1. VX Revisited

R.W. Bide
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Technical Report
DRES TR 2000-063
September 2000

National Defence Défense nationale

Canada

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In conducting the research described in this report, the investigators adhered to the "Guide to the Care and Use of Experimental Animals" published by the Canadian Council on Animal Care.

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Abstract

The estimated inhalation toxicity of VX for humans was re-examined. A literature search was conducted which identified a number of unrelated and inconsistent animal toxicity studies and some varying estimates of the human inhalation toxicity of VX. The human estimates varied between 5 and 100 mg.min/m^3 without the authors identifying the related respiratory parameters in many cases.

Nose only inhalation toxicity studies in mice, rats and guinea pigs were carried out using an NaCl aerosol with entrained VX as the toxic moiety. The observed LCt_{50} values for 12 min exposures of mice, rats and guinea pigs were 72, 67 and 30 mg.min/m^3, respectively. The mouse value agrees with the 71 mg.min/m^3 reported previously by this laboratory for 10 min nose only exposures to VX vapour. No nose only values were found for rats in the literature. The literature values for whole body toxicity in rats and mice were significantly lower than the above figures. The guinea pig value in this study was similar to the 10 min, whole body value of 29 mg.min/m^3 obtained from the literature. The present values and the literature values for goats, dogs, rabbits, guinea pigs (which represent a mixture of whole body vapour and aerosol exposures at varying times) and the one nose only value for mice were combined with "standard" minute-volume/body weight data to provide an estimate of human toxicity of 7 mg.min/m^3 for a 70 kg human breathing 15 L/min for 10 min. This is the best estimate that can be made from the somewhat disparate animal data currently available. It is recommended that the CF adopt this 7 mg.min/m^3 value for inhalation toxicity of VX and that the value by reviewed regularly to incorporate any new information that becomes available. Differences in the exposure times, body weights and breathing rates will produce corresponding changes in the toxicity value.

Résumé

On a réexaminé la toxicité par inhalation estimative du VX chez l'homme. Une recherche documentaire a permis de relever un certain nombre d'études toxicologiques chez l'animal sans rapport et sans cohérence, ainsi que quelques estimations distinctes de la toxicité par inhalation du VX chez l'homme. Les estimations pour l'homme variaient entre 5 et 100 mg.min/m^3, sans que les auteurs aient identifié les paramètres respiratoires connexes dans bien des cas.

Des études sur la toxicité liée à l'inhalation par le nez seulement chez la souris, le rat et le cobaye ont été effectuées avec un aérosol de chlorure de sodium (NaCl) et du VX entraîné comme partie toxique. Les valeurs CLt_{50} observées pour des expositions de 12 min chez la souris, le rat et le cobaye étaient de 72, 67 et 30 mg.min/m^3 respectivement. La valeur observée chez la souris concorde avec les 71 mg.min/m^3 signalés précédemment par ce laboratoire pour des expositions de 10 min à des vapeurs de VX par le nez seulement. On n'a trouvé aucune valeur se rapportant à l'inhalation par le nez seulement chez le rat dans la littérature. Les valeurs fournies dans la littérature concernant la toxicité pour le corps entier chez le rat et la souris étaient sensiblement inférieures aux valeurs ci-dessus. La valeur relative au cobaye dans cette étude est similaire à la valeur fournie dans la littérature pour 10

min et pour le corps entier, soit 29 mg min/m^3. Les valeurs actuelles et les valeurs de la littérature pour la chèvre, le chien, le lapin, le cobaye (mélange d'expositions du corps entier à de la vapeur et de l'aérosol pour des durées différentes) et l'unique valeur relative à l'inhalation par le nez seulement chez la souris ont été combinées aux données « standard » minutes-volume/poids corporel pour produire une estimation de la toxicité chez l'homme de 7 mg.min/m^3, pour un sujet de 70 kg qui respire 15 l/min pendant 10 min. C'est la meilleure estimation qui puisse être faite à par partir des données animales assez disparates actuellement disponibles. Il est recommandé que les FC adoptent cette valeur de 7 mg.min/m^3 pour la toxicité par inhalation du VX et que cette valeur soit réexaminée régulièrement pour tenir compte des nouveaux renseignements qui deviennent disponibles. Des différences dans la durée d'exposition, le poids corporel et le rythme de la respiration entraînent des changements correspondants de la valeur toxicologique.

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Executive summary

BACKGROUND

The definitive inhalation toxicity of VX is of direct interest to the CF for design of materiel, as a basis for comparison among agents and as a comparative value for non- volatile pesticides. Literature values for the human LCt_{50} of VX vary from 5 to 100 mg.min/m^3 without the necessary physiologic parameters being stated in all cases. These values are surrounded by controversy and, when the attendant scientific data were examined closely, none could be confidently supported. In addition, the animal toxicity data did not appear to follow the usual body-size related, interspecies pattern observed with other nerve agents.

In an attempt to clarify the toxicity issues, nose only inhalation studies in mice, rats and guinea pigs were carried out using VX entrained in an NaCl aerosol as the toxic moiety. The results were combined with literature values to provide an estimate of human toxicity.

RESULTS

The measured inhalation toxicities (LCt_{50}) for mice, rats and guinea pigs were 72, 67 and 30 mg.min/m^3, respectively. All of the affected subject animals exhibited similar clinical signs of organophosphate poisoning. All casualties died within 20 min of the end of the exposures. No lingering conditions were observed.

DISCUSSION

The LCt_{50} values obtained for the nose only inhalation toxicity of mice and rats were much higher that those reported for whole body exposures. The one reported value for nose only vapour exposure of mice to VX was similar to the values obtained in this study. The difference has been attributed, previously, to skin absorption of agent. However, the animals in question were not clipped or depilated so that the fur would protect the skin and, further, the authors apparently did not consider the probable ingestion of agents during grooming by these rodent species.

An estimate of human inhalation toxicity (LCt_{50}) was made using the minute-volume/body weight ratio used with GB as the interspecies comparison. The literature data for the toxicity of VX to the goat, dog, rabbit, guinea pig and nose only mouse exposures provided a linear relationship which mimics the situation found with GB. The mouse, rat and guinea pig data generated in this study supported this relationship. A human inhalation toxicity estimate was prepared using the goat, dog, rabbit, guinea pig, nose only vapour toxicity data for the mouse and the data generated in this study. Using the MV/BW values for the laboratory species and that of 0.223 for humans developed previously in this laboratory, an estimated human inhalation toxicity value of 7 mg.min/m^3 was calculated for a 70 kg human breathing 15 L/min for 10 min. This value is in agreement with that recommended by WHO [1] and similar to that given in a recent US military text [2].

CONCLUSIONS

The estimated value for the inhalation toxicity of VX is 7 mg.min/m^3 for a 10 min exposure to a 70 kg human breathing 15 L/min. Values for different times, body weights and respiration rates will vary proportionately.

RECOMMENDATION

The value of 7 mg.min/m^3 be adopted by the CF for the inhalation toxicity of VX for a 70 kg human breathing 15 L/min for 10 min. This value should be re-examined on a regular basis to incorporate additional toxicity information as it becomes available.

Note: It is very important that the body weight, respiration rate and exposure time be included whenever this value is listed because a simple concentration-time product will not predict the effects at other exposure times and changes in body weight and respiration rates will alter the toxicity value.

Bide, R.W. and Risk, D.J. (2000). Inhalation toxicity of aerosolized nerve agents 1. VX revisited. (DRES TR 2000-063). Defence Research Establishment Suffield.

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Sommaire

CONTEXTE

La toxicité par inhalation définitive du VX intéresse directement les FC pour la conception de matériel, en tant que base de comparaison entre les agents et valeur comparative pour les pesticides non volatils. Les valeurs fournies par la littérature pour le CLt_{50} du VX chez l'homme varient de 5 à 100 mg.min/m^3, sans que les paramètres physiologiques nécessaires soient indiqués dans tous les cas. Ces valeurs s'entourent d'une controverse et, en examinant de près les données scientifiques connexes, on a constaté qu'aucune ne pouvait être solidement appuyée. De plus, les données sur la toxicité chez l'animal ne semblaient pas se conformer au modèle habituel, lié à la taille corporelle et avec comparaison entre espèces, qui s'observe dans le cas des autres agents neurotoxiques.

Dans le but de clarifier les questions toxicologiques en cause, on a effectué des études d'inhalation par le nez seulement chez la souris, le rat et le cobaye en utilisant du VX entraîné dans un aérosol de NaCl servant de partie toxique. Les résultats ont été combinés aux valeurs de la littérature pour produire une estimation de la toxicité chez l'homme.

RÉSULTATS

La toxicité par inhalation (CLt_{50}) mesurée chez la souris, le rat et le cobaye était de 72, 67 et 30 mg.min/m^3 respectivement. Tous les animaux affectés présentaient de semblables signes cliniques d'empoisonnement aux organophosphorés. La mort est survenue dans les 20 minutes suivant la fin de l'exposition. Aucun trouble persistant n'a été observé.

ANALYSE

Les valeurs du CLt_{50} obtenues pour la toxicité liée à l'inhalation par le nez seulement chez la souris et le rat étaient beaucoup plus élevées que celles signalées pour les expositions du corps entier. L'unique valeur signalée pour l'exposition à des vapeurs de VX par le nez seulement chez la souris était semblable aux valeurs obtenues dans cette étude. La différence a précédemment été attribuée à l'absorption de l'agent par voie cutanée. Cependant, les animaux en question n'avaient pas été tondus ou épilés, de sorte que leur fourrure protégeait la peau, et de plus, les auteurs n'ont apparemment pas tenu compte de l'ingestion probable d'agents par ces rongeurs lorsqu'ils faisaient leur toilette.

On a estimé la toxicité par inhalation chez l'homme (CLt_{50}) à partir du ratio minute-volume/poids corporel employé avec le GB pour la comparaison entre espèces. Les données que l'on trouve dans la littérature concernant la toxicité du VX chez la chèvre, le chien, le lapin et le cobaye et les expositions par le nez seulement chez la souris donnent une relation linéaire qui s'apparente à la situation que l'on retrouve avec le GB. Les données sur la souris, le rat et le cobaye obtenues dans cette étude confirment cette relation. Une estimation de la toxicité par inhalation chez l'homme a été établie à partir des données sur la toxicité chez la chèvre, le chien, le lapin et le cobaye et l'exposition à des vapeurs par le nez seulement chez la souris, ainsi qu'à partir des données de cette étude. Utilisant les valeurs MV/PC pour les

espèces de laboratoire et celle de 0,223 pour l'homme déterminée précédemment dans de laboratoire, on a estimé que la toxicité par inhalation chez l'homme était de 7 mg.min/m^3 pour un sujet de 70 kg respirant 15 l/min pendant 10 min. Cette valeur concorde avec celle que recommande l'OMS [1] et elle est semblable à celle qui a été publiée récemment dans un texte militaire des États-Unis [2].

CONCLUSIONS

La valeur estimative de la toxicité par inhalation du VX est de 7 mg.min/m^3 pour une exposition de 10 min chez un être humain de 70 kg respirant 15 l/min. Les valeurs se rapportant à des durées d'exposition, des poids corporels et des rythmes de la respiration différents varient proportionnellement.

RECOMMANDATION

Les FC devraient adopter la valeur de 7 mg.min/m^3 pour la toxicité par inhalation du VX chez un être humain de 70 kg respirant 15 l/min pendant 10 min. Cette valeur devrait être réexaminée régulièrement pour qu'il soit tenu compte des données additionnelles sur la toxicité au fur et à mesure qu'elles deviennent disponibles.

Nota : Il est très important que le poids corporel, le rythme de la respiration et la durée d'exposition soient inclus chaque fois que cette valeur est indiquée, parce que le simple produit concentration-durée ne prédit pas les effets pour d'autres durées d'exposition et que les changements dans le poids corporel et le rythme de la respiration modifient la valeur de la toxicité.

Bide, R W and Risk, D.J. (2000). Inhalation toxicity of aerosolized nerve agents 1. VX revisited. (DRES TR 2000-063). Defence Research Establishment Suffield.

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The VX required was originally synthesized by Mr. A. Hansen (retired) and then purified by Dr. C. Boulet and Dr. R. Clewley of the Chemical Biological Defence Section, DRES.

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Introduction

In the 1990-91 Gulf War, the UN forces were faced with the possibility of nerve agent chemical attack by Iraq. This was the first time that such a threat was probable in an active theatre of operations since the discovery of these agents. One result was a intense review of the toxicological information concerning the nerve agents. When the literature concerning VX was assessed, it became apparent that the toxicity data were inconsistent, incomplete and controversial. The human estimate for the inhalation toxicity (LCt_{50}) of VX has been variously reported as 5-10 mg.min/m^3 [1], 10 mg.min/m^3 [2], 30 mg/min/m^3 [3], 100 mg.min/m^3 [4] and a controversial value of 15 mg.min/m^3 has been recommended [5]. The supporting data is piece-meal. Human exposures are mixed with animal data and neither are well documented. One major reference [2] gives no supporting evidence or even the origin of the quoted number. Further complicating the situation, some animal studies have been whole body exposures, some nose only, some to vapour, some to aerosol and in several cases, the method/means of exposure have not been chronicled.

Estimation of human inhalation toxicity from animal data requires some form of extrapolation incorporating a mechanism to adjust the toxicity values for the differences in size of the species involved [6, 7, 8]. One way to accomplish this [9, 10] is by systematic correlation of the observed toxicity with some measure of body size/function. This approach is endorsed by the USEPA [8] and appears in a number of texts [7, 11, 12].

An empirical comparison of the minute-volume/body weight ratio (MV/BW) and inhalation toxicity (LCt_{50}) provides a reasonable, if empirical, interspecies comparator to obtain a human inhalation toxicity estimate for GB (Fig. 1; [13, 14, 15]). When this comparison was applied to the available inhalation toxicity data for VX in animals (Fig. 2), the apparent relationship was very different from that obtained with GB. The differences were concerned with the whole body, inhalation toxicities [16, 17, 18] of the smaller laboratory species; mice, rats and hamsters. However, a value for mice obtained by careful nose only exposure [19], appeared to indicate that VX might follow a similar body size/respiratory relationship to GB providing that nose only, rather than whole body, exposures were carried out to minimize effects from percutaneous, ocular or oral exposure.

In the studies of VX reported here, the toxicities to mice, rats and guinea pigs of VX containing, NaCl aerosols were established, compared to the available literature values and used with an empirical relationship to estimate the human inhalation toxicity for VX.

Materials and methods

Chemicals

O-Ethyl-S-(2-diisopropylaminoethyl) methylphosphorothiolate (VX, MW 268, CAS# 50782-69-9, density 1.09 g/cc) was synthesized at the Defence Research Establishment Suffield (DRES) by Mr. A. Hansen then purified by Kuglerohr distillation prior to use. The VX was 99% pure as indicated by GC/MS and NMR.

Glucose-C^{14} was purchased from Sigma Chemical Co. St. Louis, MO. Phosphate buffered isotonic saline (Dulbecco A) was prepared as sterile solution from a prepared commercial powder mixture (Oxoid Ltd., England).

Counting methods

Radioactivity was quantified by liquid scintillation counting in a Beckman LS 6000 liquid scintillation spectrometer (Beckman Instruments Inc., Fullerton, CA) using a standard scintillation cocktail containing, in 4 L, 2680 mL toluene, 1320 mL Triton X100, 20 g diphenyl oxazole and 2 g bis-(o-methylstyryl)benzene.

Animals

The male mice, rats and guinea pigs used in this study were outbred CD®1(ICR)BR, CD®(SD)BR and (HA)BR strains, respectively, purchased from Charles River Canada, St. Constant, Que and were acclimatized for at least 7 days in the Vivarium at DRES before use.

Inhalation exposures

Source Solutions

The saline aerosols were generated from source solutions consisting of isotonic saline, marker glucose-C^{14} and appropriate amounts of VX added as solutions in 2-propanol. The generation required a minimum of 3.3 mL of saline-glucose-C^{14} to which 100 µL of VX solution was added. The glucose-C^{14} was added to the saline at the start of each day so that a 100 µL sample of the source solution would count at ≈2000 dpm.

Description of the system

The inhalation exposure system has been described in detail [20]. The usual t_{99} value for the system was between 45 and 60 sec. At 60 sec, this is 8 per cent of the 12 min exposure time and the t_{99} is within the exposure time > 13*t_{99} suggested by McFarland [21]. Under routine use, the system has 5 animals in holders mounted on one side and the off side ports are closed with rubber stoppers. The fourth port (counting from the input end) on the opposite side (fitted with a one-hole stopper) is used for monitoring the pressure differential between the inside of the chamber and the surrounding air. Before, during and after an exposure while the animals are mounted on the system, the pressure differential is continuously monitored with a centre-off Magnehelic gauge with a total range of 0.5 mm H_2O and controlled manually by adjusting the exhaust flow so that the difference is near or at zero. The centre (third) port on the off side is used to take grab samples of the exposure atmosphere.

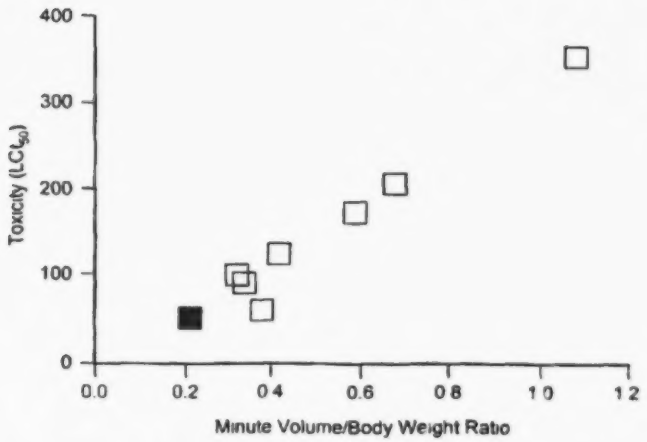

Figure 1 *Comparison of GB toxicity between species by MV/BW ratio. The values for "standard" minute volumes and body weights [32] were used to obtain the MV/BW ratio. The human value is the closed symbol. Other points, from left to right, represent monkeys, dogs, cats, guinea pigs, rats and mice.*

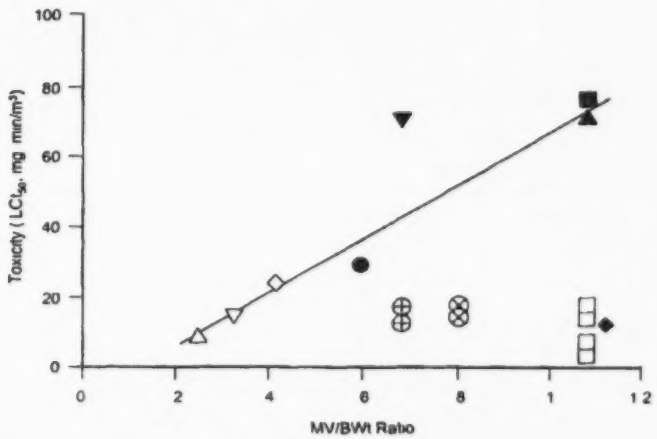

Figure 2 *Comparison of VX toxicity between species using MV/BW ratio. The open symbols represent literature values for LCt50 for mice (□), hamsters (⊗), rats (⊕) and goats (△) exposed whole body to VX for 10 min. The route of exposure for the rabbits (◇) and dogs (▽) was not stated. The data from an historical [19] nose only study in mice (▲), was considerably removed from the other mouse data. In the same study, whole body exposures produced a result (◆) comparable to other whole body mouse exposures. The toxicity values from this study for nose only aerosol exposures to mice (■), rats (▼) and guinea pigs (●), when combined with the data for larger species and the previous nose only mouse study, appear to support an allometric line (–) similar to that obtained for GB (Fig. 1).*

Animals exposures

For each species, a preliminary range finding experiment (one or two animals per exposure group) was followed by a more definitive toxicity study (5 animals per group) In all cases the exposure procedure was the same.

After the air flows had been measured and were found to be correct, the animals were placed in their holders and mounted on the sides of the chamber. When all animals were in place and the exhaust flow was adjusted to balance the air input, the run was started on the APS/computer. After 2 min to collect pre-exposure data, the aerosol generator was started by plunging the head into the source liquid. Four min later, a 3 min grab sample was started. This was repeated at 9.5 min, 7 5 min after the start of the aerosol exposure. The aerosol generator was stopped after 12 min by raising the head and turning off the air flow to the generator. After the APS indicated that the chamber had <5000 particles/L of aerosol (usually 4 min after the aerosol generation was stopped) the animals were removed from the system and returned to their cages The animals were examined daily after exposure and weighed before exposure, the day after exposure and at intervals until 14 days post exposure. A sample of the casualties were examined post-mortem for lesions when samples of lungs, trachea, liver, kidneys and brain were taken for histologic examination.

Measurement of exposure

The grab sample filters were placed in the bottom of 22 mm liquid scintillation counting vials. Ten mL of scintillation cocktail were added and, after 24 hr in the dark, the vials were counted. A 100 µL sample of the solution remaining in the generator was also mixed with 10 mL of cocktail and counted. The chemical concentration in the aerosol was then calculated from the ratio of the radioactivity on the filter, the sampling time (usually 3 min), the measured air flow through the filter and the specific activity of the source liquid. The concentration of chemical in the aerosol, in µg/L, was multiplied by the minutes of exposure and converted to $mg.min/m^3$ as the estimate of exposure. The loss of exposure during the rise to equilibrium at the start of the run was considered to be compensated by the increase in exposure as the aerosol cleared from the chamber so that no corrections were instituted for these variations. However, the particle numbers and mass were continuously monitored during the exposure and, if significant variability was encountered, that exposure was not considered in the toxicity analysis.

Data reduction and statistics

Raw particle characterization data were subjected to a "coincidence" correction routine [22] and then assembled into tables of sample number, time and particle number/mass. The particle numbers and mass were compared to the calculated aerosol concentrations indicated by the radioactivity found on the grab sample filters as a quality control measure.

Calculation of the LCt_{16}, LCt_{50} and LCt_{84} were done by both the Bliss [23] and Litchfield and Wilcoxon [24] methods using commercial programs [25, 26] and a newer process developed in this laboratory [27]. All three processes utilize PROBIT analysis but differ in the data transformations applied to values of 0 and 100 percent responses.

Comparisons of body weights were done using the Student's *t* test [28] following analysis of variance (*F* test [28]). A commercial program was used for linear regressions [26].

Results

Definition of the saline aerosol

The concentration of NaCl in the source solution determines the characteristics of the resulting aerosol [20]. A particle size spectrum taken from the middle of a typical exposure experiment (Fig. 3), shows that the majority of the particles were in the 0.5 to 2 μm range which is the respirable range for humans and laboratory animals. The geometric mean particle size was 1.07 μm. However, because of the cubic relationship between particle size and weight, the particle mass of the aerosol was in the 2 - 5 μm range with a maximum mass peak at 4 μm and two smaller mass peaks at 1.5 and 6.7 μm (Fig. 3).

Dynamics of saline exposure aerosols

The numbers of particles in the exposure aerosols increased rapidly following the starting of the generator so that within 45 sec the particles in the 0.5 - 2 μm range were at maximum and in a near stable state (Fig. 4), usually with particle numbers of ≈24,000/mL. In the 2 - 5 μm range, the particle numbers increased quickly for the first 30 sec and then more slowly over the next 2 min. In general, the particle size distribution was quasi-stable for the remainder of the 12 mir exposure period. The t_{99} of particle numbers for the system was under one min. When the aerosol generator was shut off, the particle numbers fell rapidly and exponentially for 4 min. At that time the particle numbers were <4/mL.

Toxicity of aerolized VX

Toxicity of mice

The LCt_{16}, LCt_{50} and LCt_{84} values (Table 1) obtained for aerosolized VX in mice were 28 (23:34)[1], 72.1 (45:115) and 186 (153:226), respectively for 12 min exposures. The experimental data collected for dosage, mortality and body weight effects are provided in Table 2. In all cases, the affected mice showed the typical clinical signs of organophosphate nerve agent poisoning *ie*. excess salivation with "chewing and yawning", tremors, milky lachrymation and finally convulsions before death. Times-to-death were generally short with all casualties succumbing within 20 min of the end of the exposure period. No lesions were seen on post mortem examination that were not linked to the VX poisoning. Surviving affected mice had reduced body weight for several days after exposure and all recovered and surpassed the original body weight by the end of the observation period.

[1] Values are given as LCt_{xx} (95% confidence limits)

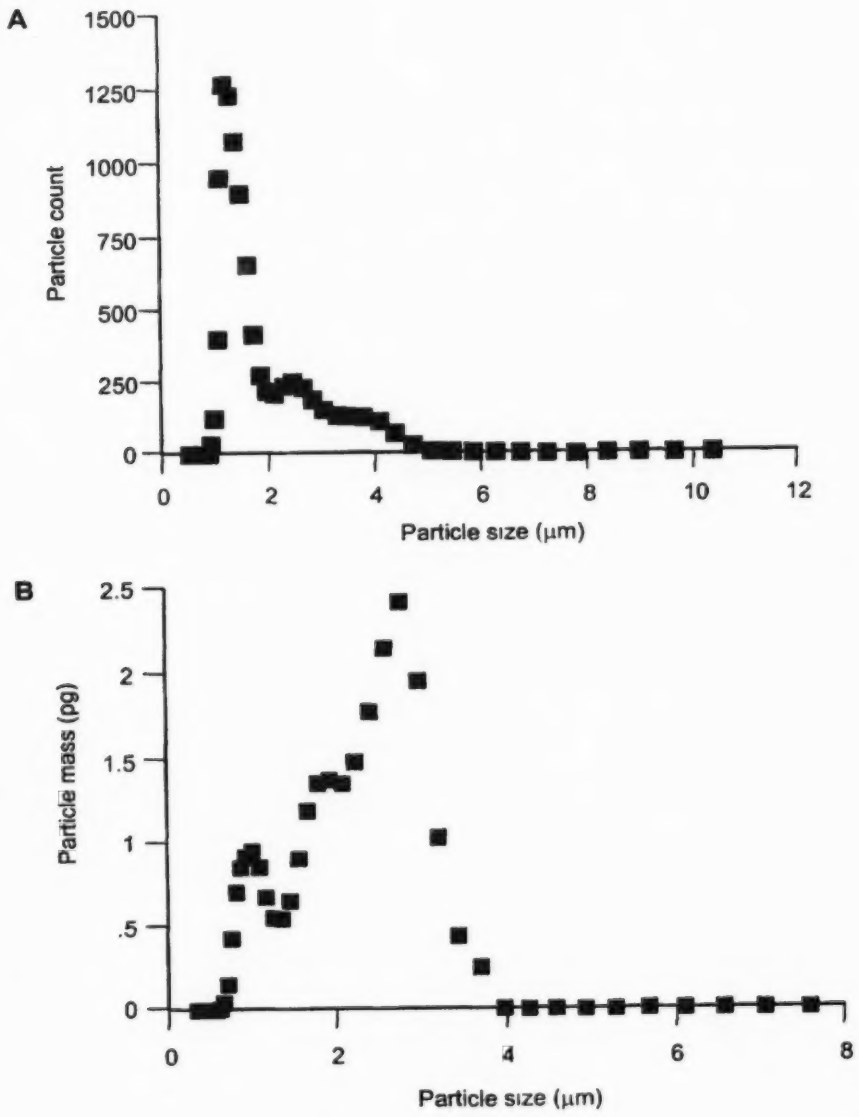

Figure 3 *Particle size and mass spectra for a saline aerosol as generated by the Lovelace generator The upper spectrum (A) shows particle numbers The geometric mean is 1 07 μm The lower spectrum (B) shows the primary size and two peaks representing particles coalesced to double and triple size*

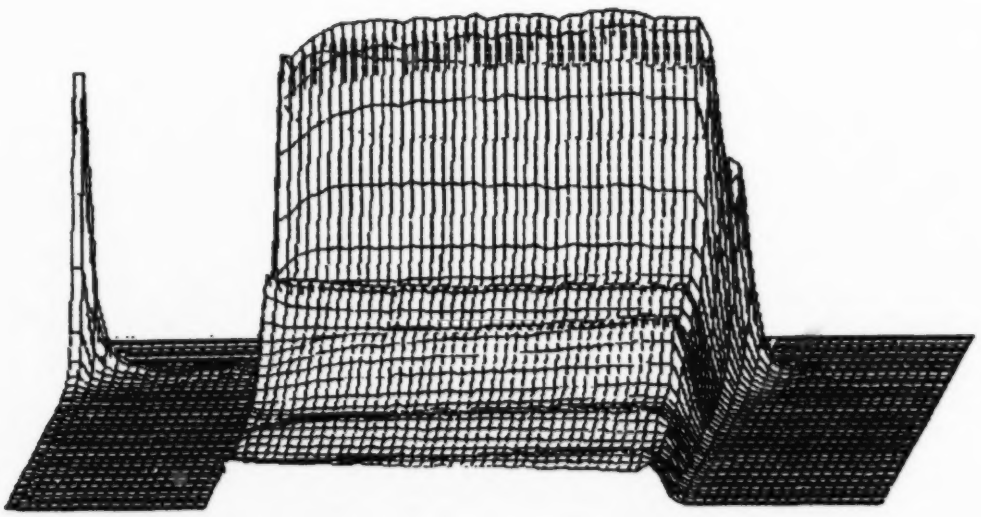

Figure 4. *Real-time 3D display of the particle size data as provided by the DYCOR software package*

Table 1. *Toxicity of VX in multiple species*

Species	Exposure		MV/BW	LCt_{50} (mg.min/m^3)		Reference
	medium	route		Measured	Calculated	
Mice	Saline aerosol	Nose only	1.076	**72.1**	73 9	This study
	Vapour	Nose only		**71**		[19]
	Vapour	Whole body		**16.1**		[19]
	Vapour[a]	Head only		**13 6**		[17]
	Aerosol[b]	Whole body[c]		**7**		[18]
	VX aerosol	?[d]		**6 1**		[16]
	Vapour[a]	Whole body		**4 0**		[17]
Rats	Saline aerosol	Nose only	0.681	**67.0**	41 9	This study
	VX aerosol	?[d]		**13 3**		[16]
	Aerosol[b]	Whole body[c]		**17**		[18]
Hamsters	Aerosol[b]	Whole body[c]	0.800[e]	**17**	52.3	[18]
	VX aerosol	?[d]		**14.7**		[16]
G pigs	Saline aerosol	Nose only	0 594	**29.6**	36.1	This study
	Aerosol[b]	Whole body[c]		**30**		[18]
	VX aerosol	?[d]		**29.5**		[16]
Rabbits	VX aerosol	?[d]	0.416	**23.9**	22 1	[16]
Dogs	VX aerosol	?[d]	0 328	**15.1**	15.3	[16]
		Whole body[c]		**15**		[18]
Goats	Vapour[a]	Whole body	0 263[e]	**9.2**	10 2	[17]

MV/BW values are suggested "standard" values [32] except where noted.

Toxicity values in bold print are those used in the human estimate regression

[a] Ten min exposures

[b] Type of aerosol not stated.

[c] The author [18] states that these data are for "Chamber exposures ." which was taken to mean whole body exposure

[d] unknown/not stated

[e] Value calculated for species from equations in [32].

Table 2. *Toxicity of aerosolized VX to mice*

Exposure (mg.min min^3)	Mortality	Body Weight Day 0 (g)	Per cent change in body weight by day				
			1	2	4	7	12
Control							
0		30 2 ±0 9	-2.1 ±3.8	1 4 ±3.8	2.9 ±3 0	4 8 ±3.4	11 3 ±5 6
Test							
5 3	0/2	29 5	-3.7	-1 1	1 6	8 4	14
70 3	1/2	30 0	-13	-12	-2 8	4.3	8 9
76	1/5	32.9 ±3 2	-1 4 ±3 0		3 0 ±1 6	4 0 ±2.1	12 ±7
79 3	2/2	27 5					
83	2/5	32 8 ±1 6	-4 5 ±5 8		1 1 ±4 0	1.9 ±3 3	15 ±3 6
113	3/5	33.2 ±2.0	-4 9		2.1	3.7	11
118	2/2	31.6					
125	3/5	34.0 ±3.4	-2.6		1.1	3.7	4 5
150	4/5	32 8 ±2 5	-9.7		-15	-11	3.0

All exposures were 12 min.

Where groups of 2 animals are indicated, the body weights are the average value For larger groups, the average body weight of surviving animals is given with the standard deviation.

Toxicity of rats

The LCt_{16}, LCt_{50} and LCt_{84} obtained (Table 1) for aerosolized VX in rats were 31 (27:34), 67 (45:99) and 146 (130:164), respectively for 12 min exposures. The experimental data collected for the exposures are given in Table 3. Times-to-death were short. All of the casualties were dead within 20 min of the end of the exposure period. Clinical signs were typical of organophosphate poisoning and post mortem examinations did not reveal any lesions that were not consistent with VX poisoning. Affected surviving animals lost body weight for several days and then regained their weight and began to grow such that they were regaining the lost weight by the end of the observation period.

Table 3. Toxicity of aerosolized VX to rats

Exposure (mg.min/min^3)	Mortality	Body Weight Day 0 (g)	Per cent change in body weight by day				
			1	2	5	8	13
Control							
0		278 ±23	0 6 ±2.4	5 9 ±2.1	24 ±8	39 ±11	43 ±11
Test							
5 3	0/2	264	0 8	3 0	18	27	33
35 2	0/5	326 ±8 6	0 6 ±0 9	2 8 ±0.4	8 1 ±1 1	8 8 ±1 7	23 ±3.4
56 5	2/5	320 ±6 4	1.6 ±1 2	2.1 ±1 5	7.7 ±2.0	8.8 ±6 9	23 ±3 3
58.3	0/5	325 ±21	-2 9 ±3 5	-1 2 ±4.4	4 4 ±3.5	8.2 ±4 1	18 ±4 1
61 5	1/5	326 ±12	-1 8 ±1.7	1 6 ±1.2	8.2 ±1 6	11 ±3 4	23 ±4 4
70 3	2/2	273					
79 3	2/2	266					
101	3/5	323 ±20	-4 6	-0.5	6 9	11	24
118	2/2	278					

All exposures were 12 min

Where groups of 2 animals are indicated, the average body weights of surviving animals are given For larger groups, the average body weight of surviving animals is given with the standard deviation

Toxicity in guinea pigs

The LCt_{16}, LCt_{50} and LCt_{84} for aerosolized VX in guinea pigs (Table 1) were 14 (12:17), 30 (19:47) and 62 (52:73), respectively for 12 min exposures. The data collected for the exposures are given in Table 4. Times-to-death were short, the majority of the casualties were dead before the purge of the exposure chamber was complete. No lingering effects were encountered. Clinical signs were typical of VX poisoning and post mortem examinations did not reveal any lesions that were not consistent with VX poisoning. Affected surviving animals lost body weight for several days and then began to grow again so that they were regaining the weight loss by the end of the observation period.

Table 4. Toxicity of aerosolized VX to guinea pigs

Exposure (mg.min min^3)	Mortality	Body Weight Day 0 (g)	Per cent change in body weight by day				
			1	2	3	8	13
Control							
0		288 ±13	1 5 ±0.8	4 3 ±0.5	6 5 ±1 2	25 ±2 0	42 ±4
Test							
5 3	0/1	292	8 8	12	9 6	18	31
18	0/5	231 ±19	0 06 ±2.9	5.1 ±2.2	11 ±3 5	23 ±4 3	34 ±6.2
24	2/5	232 ±13	-5 8 ±5.7	-2.7 ±4.3	3.2 ±4 8	15 ±6 8	28 ±14
46	3/5	239 ±13	-7 9	-5.8	-3 8	17	42
59	5/5	225 ±8					
115	5/5	224 ±39					
197	5/5	227 ±30					

All exposures were 12 min.

Where groups of 2 animals are indicated, the average body weights of surviving animals are given. For larger groups, the average body weight of surviving animals is given with the standard deviation

Discussion

The exposure system and the aerosol generation proved to be consistent and easily managed. The aerosols generated had the majority of the particles in the respiratory range of the laboratory animals (Fig. 3). The dosage estimations depend upon the assumption that the aerosol and the stock solution contain exactly the same proportion of all solutes. With this caveat, the dry NaCl aerosol will have the same proportional composition as the stock solution and the relative radioactivity of the aerosol and the stock solution may be used to estimate the amount of VX entrained in the aerosol.

These are inhalation studies. However, in addition to lung exposures, absorption of agent from particles trapped in the nasopharynx on wet tissue surfaces can also contribute significant amounts of VX to the total body dose and, hence, to the toxic responses. The upper nasal airways are the first to contact inhaled air and provide the first filter in the respiratory tract to remove particles to limit the penetration to the lower airways [29]. In the case of nerve agents, there is evidence that gaseous agents, notably G agents, are absorbed from the upper airways [30]. Aerosol particles containing agent that are trapped in the nasopharynx can transfer the agent to the tissue and to the body.

The measured toxicity (LCt_{50}) of VX in mice obtained in this study (72.1 mg.min/m^3; Table 1) was considerably higher than the values of 4 and 16 mg.min/m3 reported in the literature [16, 17, 19] for whole body vapour exposures but similar to the value of 71 mg.min/m^3 reported [19] for nose only vapour exposure Of the other literature values for mice, two were indicated as aerosol exposures and the values of 6.1 and 7 mg min/m^3, by comparison, suggest whole body exposures although no method of exposure was stated by the authors [16,18]. The remaining literature value of 13.6 mg.min/m^3 was for head only exposure [17] The values calculated from the MV/BW regression, accepted below, agree with the measured values (Table 5). The difference between whole body and nose only vapour exposures in mice has been attributed [19] to skin absorption of the agent. However, the subject mice were neither clipped nor depilated so the full hair coat was in place to protect the skin. Further, the authors appear to have ignored the extensive "lick-grooming" behaviour of mice and the ingestion of agent that would result.

As with mice, the measured toxicity for rats (Table 1) was higher than the literature values [16, 18] As no method of exposure was stated, whole body exposure was assumed as these values bear a similar relationship (whole body to nose only) to the numbers above for mice and were from the same studies. In one report [18], the author states that "Chamber exposures.." were done which, considering the state of inhalation toxicity at the time, probably indicates whole body exposures. Skin absorption may play a role in the apparent increased toxicity, but, again, the test animal exhibits "lick-grooming" behaviour and the skin would be protected by the fur so that ingestion would seem more logical. The values calculated from the accepted regression below (Table 5) are generally lower than the measured value. At this time there is not sufficient data to indicate a reason for this difference.

Table 5. *Regression values for interspecies comparisons*

Data Group	Slope	Intercept	r^2	Estimated toxicity (mg.min/m^3)				
				Mouse	Hamster	Rat	Gpig	Human
Literature data less nose only mouse	-10.7	23 2	0 178	11 7	14 6	15 9	16 8	20 8
Larger species only[a]	58 8	-4 3	0.945	59.0	42 7	35 7	30.6	8 8
Larger species plus nose only mouse from literature [19]	73 2	-10 0	0.962	68.8	48 6	39.8	33.48	6 3
Larger species plus data from this study and nose only mouse from literature	78.4	-10.5	0 864	73.9	52.3	41 9	36.1	7 0

[a] Data for goats, dogs, rabbits and guinea pigs

Human calculation for 70 kg human

Human MV/BW 0.223

LCt_{50} calculated 7 0 mg min/m^3 for human breathing 15 6 L/min 10 min

7 3 mg min/m^3 for 15 L/min breathing rate, 10 min exposure

Regression equation

LCt_{50} = 78 43638 * X - 10 45553

Literature values for hamsters [16, 18] were also much lower than the values calculated from the MV/BW vs. LCt_{50} line (Tables 1, 5; Fig.2). Again, the method of aerosol exposure was not stated but is assumed to be whole body as the numbers are from the same studies as noted above for rats and mice. Hamsters are also a grooming species so the discussions of grooming will also apply

The toxicity value for aerosol exposure of guinea pigs (Table 1) was similar to the literature value reported for vapour exposure [16, 18]. Again the method of exposure was not given in the same two studies. Although guinea pigs do groom, this species is not as active as mice and rats in this behaviour. The measured and calculated values for toxicity (Tables 1 and 5) were similar.

VX toxicities (Table 1) have been reported for several larger species [16, 17, 18]. The literature value for goats was obtained by whole body exposure. However, unlike the laboratory species, "lick-grooming" is not a common behaviour for goats so that ingestion of agent would be unlikely. The methods of exposure for the dogs and rabbits were not stated [16, 18] but again by inference are assumed to be whole body exposures. These too are not grooming species. Further, because the sources of the dog data are not clear (both articles are reviews), there may be duplication of data between studies. In the absence of evidence, however, both dog numbers were included in the calculations below.

The data presented and the process of calculating a human estimate may be criticised on several grounds. The data contain toxicity values for nose only, head only and whole body exposures and, in some cases, the modes of exposure were not given. Exposures were carried out with gas, vapour and aerosols. There are at least two exposure times. However, these are the data currently available and it is very unlikely that the experiments with large animals will be replicated in the near future. Therefore, the authors believe that the calculation presented below represents the best attempt at an human estimate that can be made at this time. As with all similar estimations, whenever more and/or newer data become available, the calculations should be redone and newer values adopted. However, it is unlikely that the addition of animal data will significantly alter the relationship between MV/BW and toxicity. Data concerning human exposures, on the other hand, may indicate a sensitivity (or lack thereof) in the human that could alter the recommended toxicity numbers significantly.

Calculation of human toxicity estimate

In the case of GB, the regression of the MV/BW ratio against LCt_{50} provides a linear, empirical relationship to describe the effects of body size upon the observed toxicity [13, 14, 15]. Similar, although less well defined, relationships may be obtained for some of the other G-type nerve agents [15, 31]. VX is also an organophosphate nerve agent with similar biological effects and it would be logical for the toxicity of VX to follow a similar empirical relationship. When the literature values for LCt_{50} of VX for goats, dogs, rabbits and guinea pigs were plotted against "standard" values [8, 32] for MV/BW, a linear relationship (Table 5; Fig. 2) was obtained that was similar to that obtained for GB. Including the nose only toxicity value data for mice from the literature [19] improved the result of the regression calculation (Table 5). The data from this study is in agreement with these calculated values. In contrast, regression of the large species data with the literature values for whole body toxicity in

rodents produces a markedly different result that does not compare with the relationship obtained for GB, has a negative rather than a positive slope to the line and is substantially different from the values obtained in this study. Thus, the data from th.. study and the literature values for rodents present a conundrum. Either, the responses to VX are markedly different from the other nerve agents or the whole body inhalation toxicity data for the rodents is compromised by some external factor.

One literature study [19] compared nose only and whole body VX exposures in mice. The whole body toxicity data obtained in this study were similar to the other whole body studies in the literature (Table 1). In contrast, the nose only toxicity values were considerably higher and compared directly with the nose only data generated in this study The authors [19] attributed the difference to percutaneous absorption of VX; *ie.* increased toxicity because of skin absorption of agent No mention was made of possible ingestion[2] of VX. However, mice, rats and hamster are species which do extensive "lick grooming" of their fur. Indeed, mice are often seen grooming during whole body chemical exposures. As VX is a sulphur containing compound that should have an affinity for hair, there is a good possibility that it would deposit on fur Certainly, fur would trap an aerosol. Any material on the fur would be licked off during grooming, taken into the mouth and absorbed during lick-grooming. The larger animals; goats, dogs, rabbits and guinea pigs; also would be subject to percutaneous absorption of VX but are also protected by their fur/hair and these non-rodent species do not "lick-groom" their coats like the rodents and would not be likely to ingest agent from that source. In support of this concept, the guinea pig values are similar for whole body and nose only exposures. Further, VX is relatively slow to cross the skin [31] which would mitigate against a significant percutaneous component combining with inhalation in a rapid lethal response. From this, the majority of the toxic response by these larger species, despite the whole body exposures, should be from inhalation whereas the response from the rodents would be a mixture of inhalation and ingestion. Thus, there is reason to doubt the validity of using whole body rodent toxicity data in the estimation of human inhalation toxicity of VX.

Using the regression line and the "standard" values for MV/BW derived previously [32, 33] for animals and the value of 0.223 for humans, a human estimate of LCt_{50} was calculated to be 7.0 mg.min/m^3 for a 70 kg human exposed for 10 min breathing 15.6 L/min (Table 5). From this number, a value of 7.3 mg min/m^3 was calculated for a 70 kg human breathing 15 L/min for 10 min. This 7 mg min/m^3 value is a quarter the value currently used in the US military manual [3] and about 1/2 that recommended by Reutter and Wade [5]. The value is within the range of values of 5-10 mg.min/m^3 quoted by the WHO group of experts [1] and similar to the 10 mg.min/m^3 value given by Sidell [2].

[2] Ingestion in this case would include intra-oral exposure. The nasopharynx is known to be a site of rapid absorption of nerve agents and it is possible that a significant proportion of agent taken into the mouth would be absorbed directly.

Conclusions

1. The best current estimate of the human inhalation toxicity of VX is 7 mg.min/m^3 for a 10 min exposure to a 70 kg human breathing 15 L/min.

2. The overall toxicity of VX delivered as a vapour or aerosol will depend upon the relative contributions of all routes of exposure *ie.* inhalation, ingestion and percutaneous exposures. It can be expected that the cumulative effect will be greater than that seen with any single route of exposure

3. The estimate of human toxicity provided here is based upon currently available animal toxicity values. As such, the estimate should be revisited and revised whenever new information becomes available. Particular emphasis should be placed upon human data or large animal toxicity values.

Recommendations

1. That the value of 7 mg.min/m^3 be adopted by the CF as the functional toxicity (LCt_{50}) value for VX for 10 min exposure of a 70 kg human breathing 15 L/min.

2. Because there is no evidence to support any exposure time other than 10-12 min, that experimental work proceed to establish the effects of exposure time upon the toxicity of VX, particularly for longer exposure times, as this information is a recognized gap in available knowledge [34].

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The estimated inhalation toxicity of VX for humans was re-examined. A literature search was conducted which identified a number of unrelated and inconsistent animal toxicity studies and some varying estimates of the human inhalation toxicity of VX. The human estimates varied between 5 and 100 mg.min/m^3 without the authors identifying the related respiratory parameters.

Nose only inhalation toxicity studies in mice, rats and guinea pigs were carried out using an NaCl aerosol with entrained VX as the toxic moiety. The observed LCt_{50} values for 12 min exposures of mice, rats and guinea pigs were 72, 67 and 30 mg min/m^3, respectively. The mouse value agrees with the 71 mg.min/m^3 reported previously by this laboratory for 10 min nose only exposures to VX vapour. No nose only values were found for rats in the literature. The literature values for whole body toxicity in rats and mice were significantly lower than the above figures. The guinea pig value in this study was similar to the 10 min, whole body value of 29 mg min/m^3 obtained from the literature. The present values and the literature values for goats, dogs, rabbits, guinea pigs (which represent a mixture of whole body vapour and aerosol exposures at varying times) and the one nose only value for mice were combined with "standard" minute-volume/body weight data to provide an estimate of human toxicity of 7 mg.min/m^3 for a 70 kg human breathing 15 L/min for 10 min. This is the best estimate that can be made from the somewhat disparate animal data currently available. It is recommended that the CF adopt this 7 mg min/m^3 value for inhalation toxicity of VX and that the value by reviewed regularly to incorporate any new information that becomes available. Differences in the exposure times, body weights and breathing rates will produce corresponding changes in the toxicity value.

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inhalation toxicity
mice
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